

Effect of Coexposure to Asbestos and Kerosene Soot on Pulmonary Drug-Metabolizing Enzyme System

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This article reports the effect of coexposure to Indian chrysotile asbestos (5 mg/rat) and kerosene soot (5 mg/rat) on the pulmonary phase I and phase II drug-metabolizing enzymes 1, 4, 8, 16, 30, 90, and 150 days after a single intratracheal inoculation. Exposure to soot resulted in a significant induction of the pulmonary microsomal cytochrome P450 and the activity of dependent monooxygenase, benzo[a]pyrene (B[a]P) hydroxylase, and epoxide hydase at all time intervals. On the other hand, the cytosolic glutathione S-transferase (GST) activity was induced at days 1, 4, 8, 16, and 30 after exposure, followed by inhibition in the enzyme activity. In contrast, chrysotile exposure depleted cytochrome P450, B[a]P hydroxylase, epoxide hydase, and GST at initial stages, while all these parameters except GST were induced at later stages. However, coexposure to chrysotile and soot led to a significant inhibition in the cytochrome P450 levels, activities of B[a]P hydroxylase, epoxide hydase, and GST at initial stages of exposure. At advanced stages, however, an additional increase in cytochrome P450, B[a]P hydroxylase, and epoxide hydase but a decrease in GST was observed. These results clearly show that the intratracheal coexposure to high levels of asbestos and kerosene soot alters the metabolic activity of the lung, which in turn may retain toxins in the system for a longer period, resulting in adverse pathological disorders. — *Environ Health Perspect* 102(Suppl 5):181–183 (1994)

Key words: asbestos, kerosene soot, drug-metabolizing enzymes, rat lung

Introduction

Occupational exposure to asbestos produces both malignant (bronchogenic carcinoma, mesothelioma) and nonmalignant (lung and pleural fibrosis) diseases in the respiratory tract (1). In India, the majority of asbestos factory workers use kerosene as a domestic fuel because of its low cost. This creates the possibility of their exposure to asbestos at the workplace and to combustion products of kerosene (soot) at home, which may create serious health problems. Earlier reports have suggested that the continuous derangement in the pulmonary drug-metabolizing enzyme systems by asbestos affects the metabolism and clearance of a variety of environmental pollutants reaching the lung (2–4). Because kerosene soot has both aliphatic and aromatic hydrocarbons (3,5), these

hydrocarbons, which would be metabolized by cytochrome P450 dependent monooxygenase, may metabolize in the presence of asbestos, so that their persistence, and hence, toxic responses in the system may be affected. In this article, we report the effect of coexposure to Indian chrysotile asbestos and kerosene soot on the pulmonary phase I and phase II drug-metabolizing enzymes after 1, 4, 8, 16, 30, 90, and 150 days of exposure.

Materials and Methods

All the chemicals and reagents used were acquired either from Sigma Chemical Company, (St. Louis, MO) or Spectrochem Pvt. Ltd. (India), and were of analytical grade. Kerosene soot was prepared by burning the commercial grade kerosene (bp 177–288°C) in a pressure stove under the conditions approximating those in a kitchen. Soot layers deposited on the porcelain crucible kept under the flame were collected and used for the inoculation. Chemical compositions of soot as well as particle size distribution of dust and soot have been reported elsewhere (3).

Male albino rats (120–150 g) were divided into four subgroups. One group received an intratracheal inoculation of 5 mg of kerosene soot in 1 ml of corn oil; the second group received 5 mg of chrysotile, administered similarly; the third group received both kerosene soot and

chrysotile. The fourth group, control rats, received only 1 ml of corn oil. Six animals from each group were sacrificed 1, 4, 8, 16, 30, 90, and 150 days after exposure by decapitation; their lungs were removed and homogenized in 0.25 M sucrose. Lung microsomal and cytosolic fractions were isolated (6).

Total cytochrome P450 (7), benzo[a]pyrene (B[a]P) hydroxylase (8), epoxide hydase (9), glutathione S-transferase (GST) (10) and protein (11) were assayed by standardized methods. The results were presented as mean \pm standard error (SE) of six animals. Statistical significance was determined by Student's *t*-test (two-tailed) and a value of $p < 0.05$ was considered significant.

Results

Exposure of kerosene soot resulted in a significant induction of microsomal cytochrome P450 in rat lungs after 1, 4, 8, 16, 30, 90, and 150 days with a maximum induction ($\approx 570\%$) at day 1, followed by a gradual loss in the induction of cytochrome P450 (Table 1). However, chrysotile exposure significantly depleted lung microsomal cytochrome P450 content on days 1 and 4 only, while after 30, 90, and 150 days an increase in the hemoprotein content was observed. Simultaneous exposure to chrysotile and soot significantly depleted cytochrome P450 in rat lungs up

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Table 1. Effect of kerosene soot and Indian chrysotile asbestos on phase I and phase II drug-metabolizing enzymes in rat lungs.

| Parameters | Posttreatment period, days | | | | | | |
|--|---|-------------------------------|----------------------------------|----------------------------------|----------------------------------|-----------------------------------|----------------------------------|
| | 1 | 4 | 8 | 16 | 30 | 90 | 150 |
| Cytochrome P450, nmole/mg protein | | | | | | | |
| Control | 0.23 ± 0.01 | 0.24 ± 0.01 | 0.19 ± 0.01 | 0.21 ± 0.01 | 0.19 ± 0.01 | 0.25 ± 0.02 | 0.28 ± 0.01 |
| Soot | 1.31 ± 0.03 ^a | 1.04 ± 0.02 ^a | 0.66 ± 0.02 ^a | 0.59 ± 0.01 ^a | 0.38 ± 0.01 ^a | 0.36 ± 0.03 ^c | 0.42 ± 0.02 ^a |
| Chrysotile | 0.17 ± 0.01 ^b | 0.20 ± 0.01 ^c | 0.17 ± 0.01(NS) | 0.20 ± 0.01 (NS) | 0.23 ± 0.01 ^c | 0.32 ± 0.01 ^c | 0.56 ± 0.02 ^a |
| Chrysotile + soot | 0.37 ± 0.01 ^{a,xx,*} | 0.41 ± 0.02 ^{a,xx,*} | 0.29 ± 0.02 ^{a,xx,*} | 0.44 ± 0.01 ^{a,xx,*} | 0.45 ± 0.02 ^{a,xxx,*} | 0.69 ± 0.02 ^{a,xx,*} | 0.65 ± 0.01 ^{a,xx,**} |
| Benzo[a]pyrene hydroxylase, fluorescence units/min/mg protein | | | | | | | |
| Control | 0.24 ± 0.03 | 0.23 ± 0.01 | 0.26 ± 0.03 | 0.20 ± 0.02 | 0.28 ± 0.02 | 0.29 ± 0.02 | 0.27 ± 0.01 |
| Soot | 0.81 ± 0.01 ^a | 0.75 ± 0.01 ^a | 0.51 ± 0.02 ^a | 0.41 ± 0.03 ^a | 0.64 ± 0.01 ^a | 0.78 ± 0.02 ^a | 0.84 ± 0.02 ^a |
| Chrysotile | 0.16 ± 0.01 ^d | 0.18 ± 0.01 ^b | 0.16 ± 0.03 ^d | 0.20 ± 0.01 (NS) | 0.40 ± 0.02 ^b | 0.77 ± 0.02 ^a | 1.02 ± 0.05 ^a |
| Chrysotile + soot | 0.27 ± 0.01(NS) [*] | 0.27 ± 0.01 ^{c,xx,*} | 0.29 ± 0.01 (NS) ^{x,**} | 0.22 ± 0.02 (NS) ^{x,**} | 0.58 ± 0.01 ^{a,xx,*} | 0.90 ± 0.01 ^{a,xx,*} | 1.22 ± 0.05 ^{a,xx,***} |
| Epoxide hydrase, fluorescence units/hr/mg protein | | | | | | | |
| Control | 0.70 ± 0.05 | 0.60 ± 0.03 | 0.69 ± 0.01 | 0.99 ± 0.06 | 0.95 ± 0.04 | 1.06 ± 0.01 | 1.13 ± 0.02 |
| Soot | 2.64 ± 0.06 ^a | 2.06 ± 0.01 ^a | 1.85 ± 0.11 ^a | 1.54 ± 0.08 ^a | 1.82 ± 0.02 ^a | 1.87 ± 0.08 ^a | 2.28 ± 0.13 ^a |
| Chrysotile | 0.55 ± 0.01 ^c | 0.51 ± 0.02 ^d | 0.61 ± 0.02 ^b | 0.92 ± 0.03 (NS) | 1.32 ± 0.02 ^a | 1.78 ± 0.06 ^a | 2.63 ± 0.05 ^a |
| Chrysotile + soot | 0.97 ± 0.05 ^{b,xx,*} | 0.86 ± 0.04 ^{a,xx,*} | 1.08 ± 0.05 ^{a,xx,*} | 1.24 ± 0.02 ^{b,xx,*} | 2.31 ± 0.01 ^{a,xx,*} | 2.41 ± 0.06 ^{a,xx,*} | 3.33 ± 0.27 ^{a,xx,****} |
| Glutathione S-transferase, nmole 1-chloro-2,4-dinitrobenzene conjugated/min/mg protein | | | | | | | |
| Control | 125 ± 0.36 | 126 ± 0.50 | 129 ± 1.83 | 125 ± 1.62 | 128 ± 0.27 | 141 ± 2.18 | 129 ± 1.62 |
| Soot | 146 ± 5.34 ^a | 151 ± 0.44 ^a | 168 ± 3.91 ^a | 172 ± 4.06 ^a | 141 ± 2.00 ^a | 129 ± 3.98 ^d | 120 ± 1.72 ^b |
| Chrysotile | 113 ± 1.43 ^a | 113 ± 1.33 ^a | 116 ± 4.97 ^c | 120 ± 4.71 (NS) | 112 ± 3.06 ^a | 120 ± 7.73 ^d | 103 ± 6.90 ^b |
| Chrysotile + soot | 140 ± 2.84 ^a (NS) [*] | 142 ± 3.24 ^{a,xxx,*} | 141 ± 4.40 ^{a,xx,**} | 151 ± 5.32 ^{a,xx,**} | 133 ± 2.29(NS) ^{xxxx,*} | 126 ± 2.98 ^b (NS) (NS) | 108 ± 1.27 ^a (NS) |

Values represent mean ± SE (N = 6). Significance compared to control: ^ap < 0.001; ^bp < 0.01; ^cp < 0.02; ^dp < 0.05; NS, not significant. Significance compared to soot: ^{*}p < 0.001; ^{**}p < 0.01; ^{***}p < 0.02; ^{****}p < 0.05. Significance compared to chrysotile: ^{*}p < 0.001; ^{**}p < 0.01; ^{***}p < 0.02; ^{****}p < 0.05.

to 16 days after exposure. There was a significant induction in the hemoprotein content at longer treatment periods as compared to soot alone. However, coexposure to chrysotile and soot increased the cytochrome P450 content in lung microsomes compared to the effect of chrysotile alone. B[a]P hydroxylase and epoxide hydrase followed a similar pattern.

In the cytosolic fraction obtained from experimental animals, there was a small but statistically significant increase in the activity of GST 1, 4, 8, and 30 days after exposure to soot followed by inhibition of that activity on days 90 and 150. Exposure to chrysotile alone showed a significant inhibition in the activity of GST at all time intervals, as did coexposure to chrysotile and soot compared to soot alone, while the enzyme activity was significantly higher after up to 30 days, compared to chrysotile alone.

Discussion

Pulmonary phase I and phase II drug metabolizing enzyme systems play an important role in both the metabolic activation and deactivation of a variety of lipophilic chemical carcinogens, rendering them hydrophilic prior to their rapid clearance from the body (12). In the initial

stages of combined exposure to chrysotile and soot, the significant decrease in the activity of B[a]P hydroxylase and depletion of cytochrome P450 levels, compared to the effect of soot exposure alone, may slow down the metabolism of procarcinogenic substances in soot in the lung, retarding the clearance of the lipophilic procarcinogens (2,3). The accumulated procarcinogens may then increase the number of fibers penetrating the epithelium, increasing their persistence. This has been observed with cigarette smoke and asbestos (13). However, in the later stages after exposure, a reverse pattern with a progressive increase in the cytochrome P450 contents was recorded, although the reason for the change in response is not clear. However, depletion in the cytochrome P450 levels and associated B[a]P hydroxylase activity may be due to the interaction of various carcinogenic substances with the microsomal membrane, which may in turn lead to destabilization of the cytochrome P450 assembly (2). The induction of cytochrome P450, as observed during the late stages of both asbestos exposure alone and in combination with soot, may be due to stimulation of transcription regulatory elements of the cytochrome P450 gene by asbestos-mediated slow release of heme

during the initial stages of exposure (2). The heme released may also be involved in the generation of reactive oxygen species which catalyzes the oxidation of 6-hydroxyB[a]P to 6-oxoB[a]P (14), thereby playing an important role in the promotion of the multistage processes of chemical carcinogenesis (15).

The increase in activity of epoxide hydrase monitored in the advanced stages of coexposure to chrysotile and soot may produce more reactive carcinogenic epoxides. Further, due to the inhibition of GST activity in the late stages, epoxides may persist for longer and may be able to react with biological macromolecules, leading to a potential increase of lung cancer (2). Additional evidence on disease development in asbestos-exposed subjects may be derived from the fact that the incidence of bronchogenic carcinoma in smoking subjects is much higher than in nonsmokers (16).

These studies in rats indicate that chrysotile, together with kerosene soot, influences the clearance mechanism of the lung by altering the activities of drug metabolizing enzymes. This results in the retention of toxic compounds in the system which may lead to multiple hits with time causing neoplastic progression.

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